

formamide. rinsing well in deionized H₂O, blowing dry, and storing at room temperature.

C. PREPARATION OF LABELED RNA/HYBRIDIZATION TO ARRAY

5 1. Tagged primers

The primers used to amplify the target nucleic acid should have promoter sequences if one desires to produce RNA from the amplified nucleic acid. Suitable promoter sequences are shown below and include:

- 10 (1) the T3 promoter sequence:

5'-CGGAATTAACCCCTCACTAAAGG

5'-AATTAACCCCTCACTAAAGGGAG;

- (2) the T7 promoter sequence:

5' TAATACGACTCACTATAGGGAG;

- 15 and (3) the SP6 promoter sequence:

5' ATTTAGGTGACACTATAGAA.

The desired promoter sequence is added to the 5' end of the PCR primer. It is convenient to add a different promoter to each primer of a PCR primer pair so that either strand may be transcribed from a single PCR product.

Synthesize PCR primers so as to leave the DMT group on. DMT-on purification is unnecessary for PCR but appears to be important for transcription. Add 25 μ l 0.5M NaOH to collection vial prior to collection of oligonucleotide to keep the DMT group on. Deprotect using standard chemistry -- 55°C overnight is convenient.

HPLC purification is accomplished by drying down the oligonucleotides, resuspending in 1 mL 0.1 M TEAA (dilute 2.0 M stock in deionized water, filter through 0.2 micron filter) and filter through 0.2 micron filter. Load 0.5 mL on reverse phase HPLC (column can be a Hamilton PRP-1 semi-prep, #79426). The gradient is 0 -> 50% CH₃CN over 25 min (program 0.2 μ mol.prep.0-50, 25 min). Pool the desired fractions, dry down, resuspend in 200 μ l 80% HAC. 30 min RT. Add 200 μ l EtOH; dry down. Resuspend in 200 μ l H₂O, plus 20 μ l NaAc pH5.5, 600 μ l EtOH. Leave 10 min on ice; centrifuge 12,000 rpm for 10 min in microfuge. Pour off supernatant. Rinse pellet with 1 mL

- EtOH, dry, resuspend in 200 μ l H₂O. Dry, resuspend in 200 μ l TE. Measure A₂₆₀, prepare a 10 pmol/ μ l solution in TE (10 mM Tris.Cl pH 8.0, 0.1 mM EDTA). Following HPLC purification of a 42 mer, a yield in the vicinity of 15 nmol from a 0.2 μ mol scale synthesis is typical.

2. Genomic DNA Preparation

- Add 500 μ l (10 mM Tris.Cl pH8.0, 10 mM EDTA, 100 mM NaCl, 2% (w/v) SDS, 40 mM DTT, filter sterilized) to the sample. Add 1.25 μ l 20 mg/ml proteinase K (Boehringer) Incubate at 55°C for 2 hours, vortexing once or twice. Perform 2x 0.5 mL 1:1 phenol:CHCl₃ extractions. After each extraction, centrifuge 12,000 rpm 5 min in a microfuge and recover 0.4 mL supernatant. Add 35 μ l NaAc pH5.2 plus 1 mL EtOH. Place sample on ice 45 min; then centrifuge 12,000 rpm 30 min, rinse, air dry 30 min, and resuspend in 100 μ l TE.

3. PCR

- PCR is performed in a mixture containing, per reaction: 1 μ l genomic DNA; 4 μ l each primer (10 pmol/ μ l stocks); 4 μ l 10 x PCR buffer (100 mM Tris.Cl pH8.5, 500 mM KCl, 15 mM MgCl₂); 4 μ l 2 mM dNTPs (made from 100 mM dNTP stocks); 1 U Taq polymerase (Perkin Elmer, 5 U/ μ l); H₂O to 40 μ l. About 40 cycles (94°C 30 sec, 55°C 30 sec, 72°C 30 sec) are performed, but cycling conditions may need to be varied. These conditions are for 0.2 mL thin wall tubes in Perkin Elmer 9600. For products in the 200 to 1000 bp size range, check 2 μ l of the reaction on a 1.5% 0.5xTBE agarose gel using an appropriate size standard. For larger or smaller volumes (20 - 100 μ l), one can use the same amount of genomic DNA but adjust the other ingredients accordingly.

4. In vitro transcription

- Mix: 3 μ l PCR product; 4 μ l 5x buffer; 2 μ l DTT; 2.4 μ l 10 mM rNTPs (100 mM solutions from Pharmacia); 0.48 μ l 10 mM fluorescein-UTP (Fluorescein-12-UTP, 10 mM solution, from Boehringer Mannheim); 0.5 μ l RNA polymerase (Promega T3 or T7 RNA polymerase); and add H₂O to 20 μ l. Incubate at 37°C for 3

h. Check 2 μ l of the reaction on a 1.5% 0.5xTBE agarose gel using a size standard. 5x buffer is 200 mM Tris pH 7.5, 30 mM $MgCl_2$, 10 mM spermidine, 50 mM NaCl, and 100 mM DTT (supplied with enzyme). The PCR product needs no purification and can be added directly to the transcription mixture. A 20 μ l reaction is suggested for an initial test experiment and hybridization; a 100 μ l reaction is considered "preparative" scale (the reaction can be scaled up to obtain more target). The amount of PCR product to add is variable; typically a PCR reaction will yield several picomoles of DNA. If the PCR reaction does not produce that much target, then one should increase the amount of DNA added to the transcription reaction (as well as optimize the PCR). The ratio of fluorescein-UTP to UTP suggested above is 1:5, but ratios from 1:3 to 1:10 - all work well. One can also label with biotin-UTP and detect with streptavidin-FITC to obtain similar results as with fluorescein-UTP detection.

For nondenaturing agarose gel electrophoresis of RNA, note that the RNA band will normally migrate somewhat faster than the DNA template band, although sometimes the two bands will comigrate. The temperature of the gel can effect the migration of the RNA band. The RNA produced from *in vitro* transcription is quite stable and can be stored for months (at least) at -20°C without any evidence of degradation. It can be stored in unsterilized 6xSSPE 0.1% triton X-100 at -20°C for days (at least) and reused twice (at least) for hybridization, without taking any special precautions in preparation or during use. RNase contamination should of course be avoided. When extracting RNA from cells, it is preferable to work very rapidly and to use strongly denaturing conditions. Avoid using glassware previously contaminated with RNases. Use of new disposable plasticware (not necessarily sterilized) is preferred, as new plastic tubes, tips, etc., are essentially RNase free. Treatment with DEPC or autoclaving is typically not necessary.

5. Fragmentation

Heat transcription mixture at 94 degrees for forty min.
The extent of fragmentation is controlled by varying Mg^{2+}
concentration (30 mM is typical), temperature, and duration of
5 heating.

6. Hybridization, Scanning, & Stripping

A blank scan of the slide in hybridization buffer only is
helpful to check that the slide is ready for use. The buffer
is removed from the flow cell and replaced with 1 mL of
10 (hydrolysed) RNA in hybridization buffer and mixed well.
Incubate for 15 - 30 min at 18°C. Remove the hybridization
solution, which can be saved for subsequent experiments.
Rinse the flow cell 4 - 5 times with fresh changes of 6 x SSPE
/ 0.1% Triton X-100, equilibrated to 18°C. The rinses can be
15 performed rapidly, but it is important to empty the flow cell
before each new rinse and to mix the liquid in the cell
thoroughly. A series of scans at 30 min intervals using a
hybridization temperature of 25°C yields a very clear signal,
usually in at least 30 min to two hours, but it may be
20 desirable to hybridize longer, i.e., overnight. Using a laser
power of 50 μ W and 50 μ m pixels, one should obtain maximum
counts in the range of hundreds to low thousands/pixel for a
new slide. When finished, the slide can be stripped using
warm water.

25 These conditions are illustrative and assume a probe
length of ~15 nucleotides. The stripping conditions suggested
are fairly severe, but some signal may remain on the slide if
the washing is not stringent. Nevertheless, the counts
remaining after the wash should be very low in comparison to
30 the signal in presence of target RNA. In some cases, much
gentler stripping conditions are effective. The lower the
hybridization temperature and the longer the duration of
hybridization, the more difficult it is to strip the slide.
Longer targets may be more difficult to strip than shorter
35 targets.

7. Amplification of Signal

A variety of methods can be used to enhance detection of
labelled targets bound to a probe on the array. In one

embodiment, the protein MutS (from *E. coli*) or equivalent proteins such as yeast MSH1, MSH2, and MSH3; mouse Rep-3, and *Streptococcus* Hex-A, is used in conjunction with target hybridization to detect probe-target complex that contain
5 mismatched base pairs. The protein, labeled directly or indirectly, can be added to the chip during or after hybridization of target nucleic acid, and differentially binds to homo- and heteroduplex nucleic acid. A wide variety of dyes and other labels can be used for similar purposes. For
10 instance, the dye YOYO-1 is known to bind preferentially to nucleic acids containing sequences comprising runs of 3 or more G residues.

8. Detection of Repeat Sequences

15 In some circumstances, *i.e.*, target nucleic acids with repeated sequences or with high G/C content, very long probes are sometimes required for optimal detection. In one embodiment for detecting specific sequences in a target nucleic acid with a DNA chip, repeat sequences are detected as
20 follows. The chip comprises probes of length sufficient to extend into the repeat region varying distances from each end. The sample, prior to hybridization, is treated with a labelled oligonucleotide that is complementary to a repeat region but shorter than the full length of the repeat. The target
25 nucleic is labelled with a second, distinct label. After hybridization, the chip is scanned for probes that have bound both the labelled target and the labelled oligonucleotide probe; the presence of such bound probes shows that at least two repeat sequences are present.

30 While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made
35 without departing from the true scope of the invention. All publications and patent documents cited in this application are incorporated by reference in their entirety for all

purposes to the same extent as if each individual publication or patent document were so individually denoted.

Mutation	Exon	Ex Size	Pop. Freq	Location	Sequence Around Mutation Site	PMID#	amp. #
297-3 C>T	1	109	marshmower	Sub G-1 > 3, Exon 3	CTTTTTCATCTTTTGGC>TAGAGAACTGGGATAGA	787/788	287
R75Q	2	109	marshmower	Substitute G-6A at 80	TATTCGCTTGGCGCG>AATGTTTTCCTTGGG	787/788	287
300 del A	1	109	marshmower	Deletion A at 4	ATCTTCTTCAGAGAACTGGGATAGAGAGCTGGCT	787/788	287
E60Y	1	109	marshmower	Substitute G-1T at 14	GAATGGGATAGAG>TAGCTGCTCTTCAAG	787/788	287
L88S	2	109	marshmower	Substitute T>C at 89	CTATGGAATCTTTTTCATATTAGGGGTAAAG	787/788	287
G86E	2	109	3.10%	Substitute G-4A at 89	TTATGTTCTATGCGAATGCTTTTATATTAG	787/788	287
R117C	4	216	0.80%	Substitute G-6A at 77	AACAGGAGGAGGACG>AGCTCTATGGGATTTAT	851/788	361
R117C	4	216	nil	Substitute C-1T at 78	AACAGGAGGAGGACG>TGCTCTATGGGATTTAT	851/788	361
Y122X	4	216	0.30%	Substitute T>A at 83	TATTCGCGATTAT>AAGTACGGCATAGGCTATTG	851/788	361
1148T	4	216	Fr Can (10%)	Substitute T>C at 170	GGGCTTCATCAGATG>TCGGATTCAGATAGAG	851/788	361
621-1 G>T	4	216	1.30%	Sub G-1T after last base	GATTTATAGAGAGG>TTAATAGCTTCCTTCAG	851/788	361
711-1 G>T	5	30	0.90%	Sub G-1T after last base	CAAAATTTGATGAAG>TTATGCTAGCTATTGAT	787/888	289
L208W	6a	164	Fr Can (10%)	Substitute T>G at 38	TGGATGGCTGCTTCTT>GGCAGGTGGCACTGCTC	934/835	331
1138 ins G	7	247	marshmower	Insert G at 137	AATCATCTCTGGGAAAGATATTGACGACATCT	788/780	404
1154 ins TC	7	247	marshmower	Insert TC at 153	TATTGACGACATCTGCA>TTCTGTCACTCTGTT	788/780	404
1161 del C	7	247	marshmower	Deletion C at 180	CGACCATCTCATCTGAT>TTGTTCTGGGACATG	788/780	404
R334W	7	247	0.40%	Substitute C-1T at 131	AAGGAATCATCTGCTC>TGGAAAGAAATTTCTTA	788/780	404
R347H	7	247	0.10%	Substitute G-6A at 171	CTGACATGTTCTGCG>AGCATGGGGGCTCAGTG	788/780	404
R347L	7	247	nil	Substitute G-1T at 171	CTGACATGTTCTGCG>TCATGGGGGCTCAGTG	788/780	404
R347P	7	247	0.50%	Substitute G-1C at 171	CTGACATGTTCTGCG>GCATGGGGGCTCAGTG	788/780	404
1078 delT	7	247	1.10%	Deletion T at 77	CTTCTCTCAGGGTCTT>TGTTGCTGTTTCTTTC	788/780	404
1248-1 G>A	7	247	marshmower	Sub G-1A after Exon 7	AACCAAAATACAGCG>ATTAATAGTACGATATG	788/780	404
A455E	8	183	0.40%	Substitute G-6A at 155	AGAGCACTTTTGGC>AGGTTCTGCGATGCA	881/882	386
G480C	10	92	nil	Substitute G > T at 46	GGAGCCTTCAGAGAG>TTGTAAMATTAAGCACA	780/880	304
G483X	10	92	0.30%	Substitute C-1T at 46	TCATTTCTGTTCTC>TAGTGTTCCTGGATAT	780/880	304
D507	10	192	0.50%	Deletion 126, 127, 128	ATTAAGAAATAATATC>TTGTTTGGTTTTCCTATG	780/880	304
F508C	10	192	0.10%	Substitute T>G at 131	TAAAGAAATATCATCT>TTGTTTGGTTTTCCTATG	780/880	304
DF508	10	192	87.20%	Deletion 129, 130, 131	ATTAAGAAATAATATC>TTGTTTGGTTTTCCTATG	780/880	304
V520F	10	192	0.20%	Substitute G-1T at 186	TAGATACAGAAAGAT>TTTCTCAAAAGCATGCC	780/880	304
1717-1 G>A	110	95	1.10%	Sub G-6A at 1-1, Exon 1	TATTTTGGTAAATAG>AGACATCTCGAAGTTT	782/783	233
G542X	11	95	3.40%	Substitute G-1T at 40	ACAATATAGTCTCT>GCGTGAAGAGTGGAAAT	782/783	233
S541N	11	95	nil	Substitute G-1T at 62	ATTAAGAAATAATATC>TTGTTTGGTTTTCCTATG	782/783	233
S541I	11	95	nil	Substitute G-1T at 62	AGGTGGATATCAGATG>TTGTTTGGTTTTCCTATG	782/783	233
S541R(A>C)	11	95	nil	Substitute A>C at 61	AGGTGGATATCAGATG>TTGTTTGGTTTTCCTATG	782/783	233
S541R(T>G)	11	95	0.30%	Substitute T>G at 63	AGGTGGATATCAGATG>TTGTTTGGTTTTCCTATG	782/783	233
G551D	11	95	2.40%	Substitute G-6A at 68	ATCACACTGAGTGGAGG>ATCAAGAGAGCAAGA	782/783	233
G551S	11	95	nil	Substitute G-6A at 67	ATCACACTGAGTGGAGG>ATCAAGAGAGCAAGA	782/783	233
G553X	11	95	nil	Substitute C-1T at 70	AGCATGAGTGGAGGCT>CTCAAGAGAGCAAGAAT	782/783	233
R553Q	11	95	nil	Substitute G-6A at 74	TGAGTGGAGTGGAGG>ATCAAGAGAGCAAGAAT	782/783	233
R553X	11	95	1.30%	Substitute C-1T at 73	TGAGTGGAGTGGAGG>ATCAAGAGAGCAAGAAT	782/783	233
A558T	11	95	nil	Substitute G-6A at 81	CGAAGAAATTTCTT>AGAGAGAGAGCAAGAAT	782/783	233
R560T	11	95	0.40%	Substitute G-6A at 85	AATTTCTTTAGCAAG>AGTGAATAGCAAT	782/783	233
R560K	11	95	nil	Substitute G-6A at 85	GAATTTCTTTAGCAAG>AGTGAATAGCAAT	782/783	233
1698-1 G>A	112	95	0.90%	Sub G-6A after last Exon 12	GAATATTTGAAGAGG>ATATATGCTTTGAAAT	831/882	289
D848V	13	724	Nil Am (83%)	Substitute A>T at 177	AAGTCTGCGGATGGA>TTTCTTTGGGAGCAAT	858/884	360
2184 ins A	13	724	2.70%	Deletion A at 286	GACAGAAACAAAAA>CAATCTTTTAAACAGAG	858/884	360
2184 ins A	13	724	nil	Insert A after 286	GACAGAAACAAAAA>CAATCTTTTAAACAGAG	858/884	360
2789-5G>A	1140	38	1.10%	Sub G-6A 5 one after last	CTCTCTGGAAATGAGG>ATATTCGATCTGCTA	888/888	374
3272-26A>G	117a	228	nil	Sub A>G 26 before 17b	TTATGTTTATTTGCAAGG>TTTCTTATGGAAA	782/801	414
3272-41T>G	117a	228	0.80%	Sub T>G 41 before 17b	TATTTGTTATGATTA>TTGTTCTAATTTAGTTCTT	782/801	414
R1066C	77	228	1.70%	Substitute G-1T at 57	AGGACATGAGCAGCTT>TTGTTCTTAAAGAGCT	782/801	414
L1077P	77b	228	nil	Substitute T>C at 81	TTACTTTGAAGCTCT>CTGTTCCAGAAAGCTC	782/801	414
Y1092X	77b	228	0.50%	Substitute G-6A at 137	CCAGCTGCTTCTTGT>AGCACTGTGAGCACTGG	782/801	414
M1101X	77b	228	nil (85%)	Substitute T>A at 163	TGGCTTGGTTTCCAA>ATNAGAGAGATAGAAATGAT	782/801	414
R1182X	78	248	3.80%	Substitute C-1T at 16	ATGGGATCTGTGAGGCG>TGAGCTTTTAAGTCT	784/785	386
3658 del C	78	248	2.80%	Deletion C at 58	AAGGTAACAGTACAGT>TTGAGCTTTTAAGTCT	784/785	386
3649-A A>G	79	248	0.0%	Sub A>G 4 after last base	TCTTGGGACAGGCTTGAGGGA>TTTGACAGCT	784/785	386
3649-10Xb	79	10Xb	40%	Sub G-1T EcoRI Fragment	ATAAATTTGGCG>TGAGTAAAGCA	79/271	450
M1282R	20	156	nil	Substitute T>C at 127	AATAGACTTTCAGAGG>TTGAGGAGGAAAGGCTTT	784/786	351
M1282X	20	156	3.10%	Substitute G-1T at 129	AATAGACTTTCAGAGG>TTGAGGAGGAAAGGCTTT	784/786	351
3905delT	20	156	2.10%	Insert T at 96	CTTGTTTATCAGCT>TTTTCAGAGCTATCAGAGC	784/786	351
4005-1 G>A	20	156	marshmower	Sub G-6A after Exon 20	ATGATGACACAGG>TTGAGGAGGAAAGGCTTT	784/786	351
N1303K	21	92	80%	Substitute C-1G at 36	CATTAGAAAAAAGG>GTTTGGATTCCTATGAG	788/783	396
N1303P	21	92	nil	Substitute A>G at 34	CATTAGAAAAAAGG>GTTTGGATTCCTATGAG	788/783	396

Table 6

WHAT IS CLAIMED IS:

General tiling claims

- 1 1. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least two sets of
3 oligonucleotide probes,
 - 4 (1) a first probe set comprising a plurality of
5 probes, each probe comprising a segment of at least three
6 nucleotides exactly complementary to a subsequence of the
7 reference sequence, the segment including at least one
8 interrogation position complementary to a corresponding
9 nucleotide in the reference sequence,
 - 10 (2) a second probe set comprising a corresponding
11 probe for each probe in the first probe set, the corresponding
12 probe in the second probe set being identical to a sequence
13 comprising the corresponding probe from the first probe set or
14 a subsequence of at least three nucleotides thereof that
15 includes the at least one interrogation position, except that
16 the at least one interrogation position is occupied by a
17 different nucleotide in each of the two corresponding probes
18 from the first and second probe sets;
19 wherein the probes in the first probe set have at least
20 two interrogation positions respectively corresponding to each
21 of two contiguous nucleotides in the reference sequence.

- 1 2. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least four sets of
3 oligonucleotide probes,
 - 4 (1) a first probe set comprising a plurality of
5 probes, each probe comprising a segment of at least three
6 nucleotides exactly complementary to a subsequence of the
7 reference sequence, the segment including at least one
8 interrogation position complementary to a corresponding
9 nucleotide in the reference sequence,
 - 10 (2) second, third and fourth probe sets, each
11 comprising a corresponding probe for each probe in the first
12 probe set, the probes in the second, third and fourth probe
13 sets being identical to a sequence comprising the
14 corresponding probe from the first probe set or a subsequence

15 of at least three nucleotides thereof that includes the at
16 least one interrogation position, except that the at least one
17 interrogation position is occupied by a different nucleotide
18 in each of the four corresponding probes from the four probe
19 sets.

1 3. The oligonucleotide array of claim 2, further
2 comprising a fifth probe set comprising a corresponding probe
3 for each probe in the first probe set, the corresponding probe
4 from the fifth probe set being identical to a sequence
5 comprising the corresponding probe from the first probe set or
6 a subsequence of at least three nucleotides thereof that
7 includes the at least one interrogation position, except that
8 the at least one interrogation position is deleted in the
9 corresponding probe from the fifth probe set.

1 4. The oligonucleotide array of claim 2, further
2 comprising a sixth probe set comprising a corresponding probe
3 for each probe in the first probe set, the corresponding probe
4 from the sixth probe set being identical to a sequence
5 comprising the corresponding probe from the first probe set or
6 a subsequence of at least three nucleotides thereof that
7 includes the at least one interrogation position, except that
8 an additional nucleotide is inserted adjacent to the at least
9 one interrogation position in the corresponding probe from the
10 first probe set.

1 5. The array of claim 2, wherein the first probe set has
2 at least three interrogation positions respectively
3 corresponding to each of three contiguous nucleotides in a
4 reference sequence.

1 6. The array of claim 2, wherein the first probe set has
2 at least 50 interrogation positions respectively corresponding
3 to each of 50 contiguous nucleotides in a reference sequence.

1 7. The array of claim 1 or 2, wherein the first probe
2 set has at least 100 interrogation positions respectively

3 corresponding to each of 100 contiguous nucleotides in a
4 reference sequence.

1 8. The oligonucleotide array of claim 1 or 2, wherein
2 the first probe set has an interrogation position
3 corresponding to each of at least 30% of the nucleotides in a
4 reference sequence and the reference sequence comprises at
5 least 100 nucleotides.

1 9. The oligonucleotide array of claim 8, wherein the
2 first probe set comprises probes which completely span the
3 reference sequence, which probes relative to the reference
4 sequence, overlap one another in sequence.

1 10. The oligonucleotide array of claim 9, wherein the
2 first probe set has an interrogation position corresponding to
3 each of the nucleotides in the reference sequence.

1 11. The oligonucleotide array of claim 10, wherein the
2 probes are oligodeoxyribonucleotides.

1 12. The oligonucleotide array of claim 1 or 2, wherein
2 the array comprises between 100 and 10,000 probes.

1 13. The oligonucleotide array of claim 1 or 2, wherein
2 the array comprises between 10,000 and 100,000 probes.

1 14. The oligonucleotide array of claim 1 or 2, wherein
2 the array comprises between 100,000 and 10,000,000 probes.

1 15. The oligonucleotide array of claim 1 or 2, wherein
2 the probes are linked to the support via a spacer.

1 16. The oligonucleotide array of claim 1 or 2, wherein
2 the segment in each probe of the first probe set that is
3 exactly complementary to the subsequence of the reference
4 sequence is 9-21 nucleotides.

1 17. The oligonucleotide array of claim 16, wherein the
2 segment is n nucleotides long, and the subsequence is at least
3 n-2 nucleotides long.

1 18. The oligonucleotide array of claim 1 or 2, wherein
2 each probe of the first probe set consists of the segment that
3 is exactly complementary to the subsequence of the reference
4 sequence.

1 19. The oligonucleotide array of claim 1 or 2, wherein
2 the probes in the second, third and fourth probe sets are
3 identical to the corresponding probe from the first probe set
4 except that the at least one interrogation position is
5 occupied by a different nucleotide in each of the four
6 corresponding probes from the four probe sets.

1 20. The array of claim 2, further comprising fifth,
2 sixth and seventh probe sets, wherein:
3 the segment of each probe in the first set
4 includes at least two interrogation positions each
5 corresponding to a nucleotide in the reference sequence,
6 the second, third and fourth probe sets, each
7 comprise a corresponding probe for each probe in the first
8 probe set, the corresponding probes in the second, third and
9 fourth probe sets being identical to a sequence comprising the
10 corresponding probe from the first probe set or a subsequence
11 of at least three nucleotides thereof that includes a first
12 interrogation position except that the first interrogation
13 position is occupied by a different nucleotide in each of the
14 four corresponding probes from the four probe sets;
15 the fifth, sixth and seventh probe sets, each
16 comprising a corresponding probe for each probe in the first
17 probe set, the probes in the fifth, sixth and seventh probe
18 sets being identical to a sequence comprising the
19 corresponding probe from the first probe set or a subsequence
20 of at least three nucleotides thereof that includes a second
21 interrogation position, except that the second interrogation

22 position is occupied by a different nucleotide in each of the
23 four corresponding probes from the four probe sets.

1 21. The array of claim 2, wherein each probe in the
2 first probe set further comprises a second segment of at least
3 three nucleotides exactly complementary to a second
4 subsequence of the reference sequence, and the probes from the
5 second, third and fourth probe sets comprise the corresponding
6 probe from the first probe set or a subsequence thereof
7 comprising the first and second segments except in the at
8 least one interrogation position.

1 22. The array of claim 2, further comprising:
2 a fifth probe set comprising at least one probe
3 comprising a segment of at least seven nucleotides exactly
4 complementary to a subsequence of the reference sequence
5 except at one or two positions, the segment including at least
6 one interrogation position corresponding to a nucleotide in
7 the reference sequence not at the one or two positions;
8 sixth, seventh and eighth probe sets, each comprising a
9 probe for each probe in the fifth probe set, the corresponding
10 probes from the sixth, seventh & eighth probe sets being
11 identical to a sequence comprising the corresponding probe
12 from the fifth probe set or a subsequence of at least nine
13 nucleotides thereof including the at least one interrogation
14 position and the one or two positions, except in the at least
15 one interrogation position, which is occupied by a different
16 nucleotide in each of the four probes.

1 23. The array of claim 2, wherein the probes are
2 arranged on the substrate so that the first set of probes is
3 arranged in a row across the substrate in an order reflecting
4 the overlap between the probes and the reference sequence, and
5 the additional sets of probes are arranged in columns relative
6 to the probes in said first set, so that probes with the same
7 interrogation position are in the same column and so that each
8 column comprises at least 4 probes.

1 24. The array of Claim 2, wherein said probes are 12 to
2 17 nucleotides in length.

1 25. The array of Claim 2, wherein said probes are 15
2 nucleotides in length and attached by a covalent linkage to a
3 site on a 3'-end of said probes, and said interrogation
4 position is located at position 7, relative to the 3'-end of
5 said probes.

1 26. The array of claim 2, further comprises fifth,
2 sixth, seventh and eighth probe sets,

3 (1) a fifth probe set comprising a plurality of
4 probes, each probe comprising a segment of at least three
5 nucleotides exactly complementary to a subsequence of a second
6 reference sequence, the segment including at least one
7 interrogation position complementary to a corresponding
8 nucleotide in the reference sequence,
9 (2) the sixth, seventh, and eighth probe sets, each
10 comprising a corresponding probe for each probe in the fifth
11 probe set, the probes in the sixth, seventh and eighth probe
12 sets being identical to a sequence comprising the
13 corresponding probe from the fifth probe set or a subsequence
14 of at least three nucleotides thereof that includes the at
15 least one interrogation position, except that the at least one
16 interrogation position is occupied by a different nucleotide
17 in each of the four corresponding probes from the fifth,
18 sixth, seventh and eighth probe sets.

1 27. The array of claim 22, wherein the first, second,
2 third and fourth probe sets have probes of a first length and
3 the fifth, sixth, seventh and eighth probe sets have probes of
4 a second length different from the first length.

Tiling for wildtype and mutant reference sequences

1 28. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least one pair of first
3 and second probe groups, each group comprising a first and
4 second sets of oligonucleotide probes as defined by claim 1;

5 wherein each probe in the first probe set from the
6 first group is exactly complementary to a subsequence of a
7 first reference sequence and each probe in the first probe set
8 from the second group is exactly complementary to a
9 subsequence from a second reference sequence.

1 29. The array of claim 28, wherein the second reference
2 sequence is a mutated form of the first reference sequence.

1 30. The array of claim 28, wherein each group further
2 comprises third and fourth probe sets, each comprising a
3 corresponding probe for each probe in the first probe set, the
4 probes in the second, third and fourth probe sets being
5 identical to a sequence comprising the corresponding probe
6 from the first probe set or a subsequence of at least three
7 nucleotides thereof that includes the interrogation position,
8 except that the interrogation position is occupied by a
9 different nucleotide in each of the four corresponding probes
10 from the four probe sets.

1 31. The array of claim 30 that comprises at least five
2 pairs of first and second probe groups, wherein the probes in
3 the first probe sets from the first groups of the five pairs
4 are exactly complementary to subsequences from five different
5 respective first reference sequences.

1 32. The array of claim 30 that comprises at least forty
2 pairs of first and second probe groups, wherein the probes in
3 the first probe sets from the first groups of the forty pairs
4 are exactly complementary to subsequences from forty
5 respective first reference sequences.

Block tiling

1 33. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least a group of probes
3 comprising:

4 a wildtype probe comprising a segment of at least three
5 nucleotides exactly complementary to a subsequence of a

6 reference sequence, the segment having at least first and
7 second interrogation positions corresponding to first and
8 second nucleotides in the reference sequence,

9 a first set of three mutant probes, each identical to a
10 sequence comprising the wildtype probe or a subsequence of at
11 least three nucleotides thereof including the first and second
12 interrogation positions, except in the first interrogation
13 position, which is occupied by a different nucleotide in each
14 of the three mutant probes and the wildtype probe;

15 a second set of three mutant probes, each identical to a
16 sequence comprising the wildtype probe or a subsequence of at
17 least three nucleotides thereof including the first and second
18 interrogation positions, except in the second interrogation
19 position, which is occupied by a different nucleotide in each
20 of the three mutant probes and the wildtype probe.

1 34. The array of claim 33, wherein the segment of the
2 wildtype probe comprises 3-20 interrogation positions
3 corresponding to 3-20 respective nucleotides in the reference
4 sequence, and the array comprises 3-20 respective sets of
5 three mutant probes, each of the three probes identical to a
6 sequence comprising the wildtype probe or a subsequence
7 thereof including the 3-20 interrogation positions, except
8 that one of the 3-20 interrogation positions is occupied by a
9 different nucleotide in each of the three mutant probes and
10 the wildtype probes, the one of the 3-20 interrogation
11 positions being different in each of the 3-20 respective sets
12 of three mutant probes.

1 35. An array of probes immobilized to a solid support
2 comprising two groups of probes, each group as defined by
3 claim 33, a first group comprising a wildtype probe comprising
4 a segment exactly complementary to a subsequence of a first
5 reference sequence and a second group comprising a wildtype
6 probe comprising a segment exactly complementary to a
7 subsequence of a second reference sequence.

- 1 36. The array of claim 35, comprising at least 10-100
2 groups of probes, each comprising a wildtype probe comprising
3 a segment exactly complementary to a subsequence of at least
4 10-100 respective reference sequences.

Pooled probes

- 1 37. A method of comparing a target sequence with a
2 reference sequence, the method comprising:
3 identifying variants of a reference sequence differing
4 from the reference sequence in at least one nucleotide;
5 assigning each variant a designation,
6 providing an array of pools of probes, each pool
7 occupying a separate cell of the array, wherein each pool
8 comprises a probe comprising a segment exactly complementary
9 to each variant sequence assigned a particular designation,
10 contacting the array with a target sequence comprising a
11 variant of the reference sequence;
12 determining the relative hybridization intensities of the
13 pools in the array to the target sequence;
14 determining the target sequence from the relative
15 hybridization intensities of the pools.

- 1 38. The method of claim 37, wherein the variants are
2 assigned numbers according to an error code.

- 1 39. The method of claim 37, wherein each variant is
2 assigned a designation having at least one digit and at least
3 one value for the digit, and each pool comprise a probe
4 comprising a segment exactly complementary to each variant
5 sequence assigned a particular value in a particular digit.

- 1 40. The method of claim 39, wherein the variants are
2 assigned successive numbers in a numbering system of base m
3 having n digits, and the array comprises $n \times (m-1)$ pools of
4 probes.

1 41. The method of claim 40, wherein each pool further
2 comprises a probe comprising a segment exactly complementary
3 to the reference sequence.

Trellis tiling

1 42. A pooled probe comprising a segment exactly
2 complementary to a subsequence of a reference sequence except
3 at a first interrogation position occupied by a pooled
4 nucleotide N, a second interrogation position occupied by a
5 pooled nucleotide selected from the group of three consisting
6 of (1) M or K, (2) R or Y and (3) S or W, and a third
7 interrogation position occupied by a second pooled nucleotide
8 selected from the group, wherein the pooled nucleotide
9 occupying the second interrogation position comprises a
10 nucleotide complementary to a corresponding nucleotide from
11 the reference sequence when the second pooled probe and
12 reference sequence are maximally aligned, and the pooled
13 nucleotide occupying the third interrogation position
14 comprises a nucleotide complementary to a corresponding
15 nucleotide from the reference sequence when the third pooled
16 probe and the reference sequence are maximally aligned,
17 wherein N is A, C, G or T(U), K is G or T(U), M is A or C, R
18 is A or G, Y is C or T(U), W is A or T(U) and S is G or C.

1 43. An array of oligonucleotide probes immobilized on
2 solid support, the array comprising:
3 first, second and third cells respectively occupied by
4 first, second and third pooled probes, each pooled probe
5 comprising a segment exactly complementary to a subsequence of
6 a reference sequence except at a first interrogation position
7 occupied by a pooled nucleotide N, a second interrogation
8 position occupied by a pooled nucleotide selected from the
9 group of three consisting of (1) M or K, (2) R or Y and (3) S
10 or W, and a third interrogation position occupied by a second
11 pooled nucleotide selected from the group, wherein the pooled
12 nucleotide occupying the second interrogation position
13 comprises a nucleotide complementary to a corresponding
14 nucleotide from the reference sequence when the pooled probe

15 and the reference sequence are maximally aligned, and the
16 pooled nucleotide occupying the third interrogation position
17 comprises a nucleotide complementary to a corresponding
18 nucleotide from the reference sequence when the pooled probe
19 and the reference sequence are maximally aligned;

20 provided that one of the three interrogation
21 positions in the each of the three pooled probes is aligned
22 with the same corresponding nucleotide in the reference
23 sequence, this interrogation position being occupied by an N
24 in one of the pooled probes, and a different pooled nucleotide
25 in each of the other two pooled probes,

26 wherein N is A, C, G or T(U), K is G or T(U), M is A
27 or C, R is A or G, Y is C or T(U), W is A or T(U) and S is G
28 or C.

1 44. The array of claim 43 further comprising:
2 fourth and fifth cells respectively occupied by fourth
3 and fifth pooled probes, each pooled probe as defined by
4 claim 43,

5 wherein one of the three interrogation position in the
6 second, third and fourth pooled probes is aligned with the
7 same corresponding nucleotide in the reference sequence, this
8 interrogation position being occupied by an N in one of the
9 pooled probes, and a different pooled nucleotide in each of
10 the other two pooled probes,

11 wherein one of the three interrogation position in the
12 third, fourth and fifth pooled probes is aligned with the same
13 corresponding nucleotide in the reference sequence, this
14 interrogation position being occupied by an N in one of the
15 pooled probes, and a different pooled nucleotide in each of
16 the other two pooled probes.

1 45. The array of claim 44, wherein the pooled probes are
2 identical except at the interrogation positions.

1 46. The array of claim 44, wherein the first, second,
2 third, fourth and fifth pooled probes are exactly
3 complementary to five respective subsequences of the reference

- 4 sequences that from each other by increments of one
5 nucleotide.

Bridge tiling

- 1 47. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least four probes:
3 a first probe comprising first and second segments, each
4 of at least three nucleotides and exactly complementary to
5 first and second subsequences of a reference sequence, the
6 segments including at least one interrogation position
7 corresponding to a nucleotide in the reference sequence,
8 wherein either (1) the first and second subsequences are
9 noncontiguous, or (2) the first and second subsequences are
10 contiguous and the first and second segments are inverted
11 relative to the complement of the first and second
12 subsequences in the reference sequence;
13 second, third and fourth probes, identical to a sequence
14 comprising the first probe or a subsequence thereof comprising
15 at least three nucleotides from each of the first and second
16 segments, except in the at least one interrogation position,
17 which differs in each of the probes.
- 1 48. The array of claim 47, wherein the first and second
2 subsequences are separated by one or two nucleotides in the
3 reference sequence.

Two interrogation positions (no wildtype)

- 1 49. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least a set of four
3 probes, each of the probes comprising a segment of at least 7
4 nucleotides that is exactly complementary to a subsequence
5 from a reference sequence, except that the segment may or may
6 not be exactly complementary at two interrogation positions,
7 wherein:
8 the first interrogation position is occupied by a
9 different nucleotide in each of the four probes,
10 the second interrogation position is occupied by a
11 different nucleotide in each of the four probes,

12 in first and second probes, the segment is exactly
13 complementary to the subsequence, except at not more than one
14 of the interrogation positions, and
15 in third and fourth probes, the segment is exactly
16 complementary to the subsequence, except at both of the
17 interrogation positions.

1 50. An array of probes immobilized to a support, the
2 array comprising at least 100 sets of 4 probes, each set as
3 defined by claim 49, the probes from the at least 100 sets
4 comprising at least 100 respective segments, the segments
5 having at least 100 respective first and second interrogation
6 positions.

Helper mutations

1 51. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising a set of probes
3 comprising:
4 a first probe comprising a segment of at least 7
5 nucleotides exactly complementary to a subsequence of a
6 reference sequence except at one or two positions, the segment
7 including an interrogation position not at the one or two
8 positions;
9 second, third and fourth mutant probes, each identical to
10 a sequence comprising the wildtype probe or a subsequence
11 thereof including the interrogation position and the one or
12 two positions, except in the interrogation position, which is
13 occupied by a different nucleotide in each of the four probes.

Omission of Perfectly Matched Probe

1 52. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least two sets of
3 oligonucleotide probes,
4 (1) a first probe set comprising a plurality of
5 probes, each probe comprising a segment exactly complementary
6 to a subsequence of at least 3 nucleotides of a reference
7 sequence except at an interrogation position,

8 (2) a second probe set comprising a corresponding
9 probe for each probe in the first probe set, the corresponding
10 probe in the second probe set being identical to a sequence
11 comprising the corresponding probe from the first probe set or
12 a subsequence of at least three nucleotides thereof that
13 includes the interrogation position, except that the
14 interrogation position is occupied by a different nucleotide
15 in each of the two corresponding probes and the complement to
16 the reference sequence,
17 wherein the probes in the first probe set have at
18 least three interrogation positions respectively corresponding
19 to each of three contiguous nucleotides in the reference
20 sequence.

Methods

1 53. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:

4 (a) hybridizing the target nucleic acid to an array
5 of oligonucleotide probes immobilized on a solid support, the
6 array comprising:

7 (1) a first probe set comprising a plurality of
8 probes, each probe comprising a segment of at least three
9 nucleotides exactly complementary to a subsequence of the
10 reference sequence, the segment including at least one
11 interrogation position complementary to a corresponding
12 nucleotide in the reference sequence,

13 (2) a second probe set comprising a corresponding
14 probe for each probe in the first probe set, the corresponding
15 probe in the second probe set being identical to a sequence
16 comprising the corresponding probe from the first probe set or
17 a subsequence of at least three nucleotides thereof that
18 includes the at least one interrogation position, except that
19 the at least one interrogation position is occupied by a
20 different nucleotide in each of the two corresponding probes
21 from the first and second probe sets;

22 wherein, the probes in the first probe set have at
23 least three interrogation positions respectively corresponding

24 to each of at least three nucleotides in the reference
25 sequence, and
26 (b) determining which probes, relative to one
27 another, in the array bind specifically to the target nucleic
28 acid, the relative specific binding of the probes indicating
29 whether the target sequence is the same or different from the
30 reference sequence.

1 54. The method of claim 53, wherein the array further
2 comprises third and fourth probe sets, each comprising a
3 corresponding probe for each probe in the first probe set, the
4 probes in the second, third and fourth probe sets being
5 identical to a sequence comprising the corresponding probe
6 from the first probe set or a subsequence of at least three
7 nucleotides thereof that includes the at least one
8 interrogation position, except that the at least one
9 interrogation position is occupied by a different nucleotide
10 in each of the four corresponding probes from the four probe
11 sets.

1 55. The method of claim 54, wherein the target sequence
2 has a substituted nucleotide relative to the reference
3 sequence in at least one undetermined position, and the
4 relative specific binding of the probes indicates the location
5 of the position and the nucleotide occupying the position in
6 the target sequence.

1 56. The method of claim 54, wherein:
2 the hybridizing step comprises hybridizing the
3 target nucleic acid and a second target nucleic acid to the
4 array; and
5 the determining step comprises determining which
6 probes, relative to one another, in the array bind
7 specifically to the target nucleic acid or the second target
8 nucleic acid, the relative specific binding of the probes
9 indicating whether the target sequence is the same or
10 different from the reference sequence and whether the second

11 target sequence is the same or different from the reference
12 sequence.

1 57. The method of claim 56, wherein the target sequence
2 has a label and the second target sequence has a second label
3 different from the label.

1 58. The method of claim 56, wherein undetermined first
2 and second proportions of the first and second target
3 sequences are hybridized to the array and the specific binding
4 indicates the proportions.

1 59. The method of claim 54, further comprising:
2 (c) removing the target nucleic acid from the array;
3 (d) hybridizing a second target nucleic acid to the
4 array;
5 (e) determining which probes, relative to one another, in
6 the array bind specifically to the second target nucleic acid,
7 the relative specific binding of the probes indicating whether
8 the second target sequence is the same or different from the
9 reference sequence.

1 60. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:
4 hybridizing the target sequence to the array of
5 claim 28;
6 determining which probes in the first group,
7 relative to one another, hybridize to the target sequence, the
8 relative specific binding of the probes indicating whether the
9 target sequence is the same or different from the first
10 reference sequence;
11 determining which probes in the second group,
12 relative to one another, hybridize to the target sequence, the
13 relative specific binding of the probes indicating whether the
14 target sequence is the same or different from the second
15 reference sequence.

1 61. The method of claim 60, wherein the hybridizing step
2 comprising hybridizing the target sequence and a second target
3 sequence to the array, and the relative specific binding of
4 the probes from the first group indicates that the target is
5 identical to the first reference sequence, and the relative
6 specific binding of the probes from the second group indicates
7 that the second target sequence is identical to the second
8 reference sequence.

1 62. The method of claim 61, wherein the first and second
2 target sequences are heterozygous alleles of a gene.

Comparative hybridization

1 63. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:
4 (a) hybridizing the reference sequence to an array
5 of oligonucleotide probes immobilized on a solid support, the
6 array comprising;
7 (1) a first probe set comprising a plurality of
8 probes, each probe comprising a segment of at least 3
9 nucleotides exactly complementary to a subsequence of the
10 reference sequence except in at least one interrogation
11 position;
12 (2) a second probe set comprising a corresponding
13 probe for each probe in the first probe set, the corresponding
14 probe in the second probe set being identical to a sequence
15 comprising the corresponding probe from the first probe set or
16 a subsequence of at least three nucleotides thereof that
17 includes the at least one interrogation position, except that
18 the at least one interrogation position is occupied by a
19 different nucleotide in each of the two corresponding probes
20 from the first and second probe sets; and
21 (b) determining which probes, relative to one
22 another, in the array bind specifically to the reference
23 sequence;
24 (c) hybridizing a target sequence to the array;

25 (d) determining which probes, relative to one
26 another, in the array bind specifically to the target
27 sequence;
28 wherein the relative specific binding of the probes
29 to the reference and the target sequence indicates whether the
30 reference sequence is the same or different from the target
31 sequence.

1 64. The method of claim 63, wherein the reference
2 sequence has a first label and the second reference sequence
3 has a second label different from the first label, and steps
4 (a) and (c) are performed simultaneously.

HIV Chip

1 65. The array of claim 2, wherein the reference sequence
2 is from a human immunodeficiency virus.

1 66. The array of claim 65, wherein the reference
2 sequence is from a reverse transcriptase gene of the human
3 immunodeficiency virus.

1 67. The array of claim 66, wherein the reference
2 sequence is from a protease gene of the human immunodeficiency
3 virus.

1 68. The array of claim 66, wherein the reference
2 sequence is a full-length reverse transcriptase gene.

1 69. The array of claim 68 comprising at least 3200
2 oligonucleotide probes.

1 70. The array of claim 66, wherein the HIV gene is from
2 the BRU HIV strain.

1 71. The array of claim 66, wherein the HIV gene is from
2 the SF2 HIV strain.

1 72. The array of claim 28, wherein the reference
2 sequence is from the coding strand of a reverse transcriptase
3 gene of a human immunodeficiency virus and the second
4 reference sequence is from the noncoding strand of the reverse
5 transcriptase gene.

1 73. The array of claim 28, wherein the first reference
2 sequence is from a reverse transcriptase gene of a human
3 immunodeficiency virus and the second reference sequence
4 comprises a subsequence of the first reference sequence with a
5 substitution of at least one nucleotide.

1 74. The array of claim 73, wherein the substitution
2 confers drug resistance to a human immunodeficiency virus
3 comprising the second reference sequence.

1 75. The array of claim 28, wherein the first and second
2 reference sequences are from a reverse transcriptase gene from
3 first and second strains of a human immunodeficiency virus.

1 76. The array of claim 28, wherein the first reference
2 sequence is from a reverse transcriptase gene of a human
3 immunodeficiency virus and the second reference sequence is
4 from a 16S RNA, or DNA encoding the 16S RNA, from a pathogenic
5 microorganism.

1 77. The array of claim 28, wherein the first reference
2 sequence is from a reverse transcriptase gene of a human
3 immunodeficiency virus and the second reference sequence is
4 from a protease gene of the human immunodeficiency virus.

1 78. The method of claim 54, wherein the reference
2 sequence is from a human immunodeficiency virus.

1 79. The method of claim 78, wherein the reference
2 sequence is from a human immunodeficiency virus and the target
3 sequence is from a second human immunodeficiency virus.

1 80. The method of claim 79, wherein the target sequence
2 has a substituted nucleotide relative to the reference
3 sequence in at least one undetermined position, and the
4 relative specific binding of the probes indicates the location
5 of the position and the nucleotide occupying the position in
6 the target sequence.

1 81. The method of claim 80, wherein the target sequence
2 has a substituted nucleotide relative to the reference
3 sequence in at least one position, the substitution conferring
4 drug resistance to the human immunodeficiency virus, and the
5 relative specific binding of the probes reveals the
6 substitution.

1 82. The method of claim 78, wherein:
2 the hybridizing step comprises hybridizing the
3 target nucleic acid and a second target nucleic acid, the
4 second target sequence being from a reverse transcriptase gene
5 of a third human immunodeficiency virus, to the array; and
6 the determining step comprises determining which
7 probes, relative to one another, in the array bind
8 specifically to the target nucleic acid or the second target
9 nucleic acid, the relative specific binding of the probes
10 indicating whether the target sequence is the same or
11 different from the reference sequence and whether the second
12 target sequence is the same or different from the reference
13 sequence.

1 83. The method of claim 82, wherein the first target
2 sequence has a first label and the second target sequence has
3 a second label different from the first label.

1 84. The method of claim 82, wherein undetermined first
2 and second proportions of the first and second target
3 sequences are hybridized to the array and the specific binding
4 indicates the proportions.

CFTR Chip

1 85. The array of claim 2, wherein the reference sequence
2 is from a CFTR gene.

1 86. The array of claim 85, wherein the reference
2 sequence is exon 10 of a CFTR gene, and said array comprises
3 over 1000 oligonucleotide probes, 10 to 18 nucleotides in
4 length.

1 87. The array of claim 85, wherein said array comprises
2 a set of probes comprising a specific nucleotide sequence
3 selected from the group of sequences comprising:
4 3'-TTTATAXTAG;
5 3'- TTATAGXAGA;
6 3'- TATAGTXGAA;
7 3'- ATAGTAXAAA;
8 3'- TAGTAGXAAC;
9 3'- AGTAGAXACC;
10 3'- GTAGAAAXCCA;
11 3'- TAGAAAXCAC; and
12 3'- AGAAACXACA; wherein each set comprises 4 probes,
13 and X is individually A, G, C, and T for each set.

1 88. The array of claim 85, wherein said group of
2 sequences comprises:
3 3'-TTTATAXTAGAAACC;
4 3'- TTATAGXAGAAACCA;
5 3'- TATAGTXGAAACCAC;
6 3'- ATAGTAXAAACCACA;
7 3'- TAGTAGXAACCACAA;
8 3'- AGTAGAXACCACAAA;
9 3'- GTAGAAAXCCACAAAG;
10 3'- TAGAAAXCACAAAGG; and
11 3'- AGAAACXACAAAGGA; wherein each set comprises 4
12 probes, and X is individually A, G, C, and T for each set.

1 89. The array of claim 32, wherein the forty first
2 reference sequences are from a CFTR gene.

1 90. The array of claim 89, wherein each of the forty
2 first reference sequences includes a site of a mutation and at
3 least one adjacent nucleotide.

1 91. The array of claim 90, wherein each of the forty
2 first reference sequences comprises at least five contiguous
3 nucleotides from a CFTR gene.

1 92. The array of claim 89, wherein at least one first
2 reference sequence is a from the coding strand of the cystic
3 fibrosis gene and at least one first reference sequence is
4 from the noncoding strand of the CFTR gene.

1 93. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least a group of probes
3 comprising:

4 a wildtype probe exactly complementary to a subsequence
5 of a reference sequence from a cystic fibrosis gene, the
6 segment having at least five interrogation positions
7 corresponding to five contiguous nucleotides in the reference
8 sequence,

9 a first set of three mutant probes, each identical to the
10 wildtype probe, except in a first of the five interrogation
11 positions, which is occupied by a different nucleotide in each
12 of the three mutant probes and the wildtype probe;

13 a second set of three mutant probes, each identical to
14 the wildtype probe, except in a second of the five
15 interrogation positions, which is occupied by a different
16 nucleotide in each of the three mutant probes and the wildtype
17 probe;

18 a third set of three mutant probes, each identical to the
19 wildtype probe, except in a third of the five interrogation
20 positions, which is occupied by a different nucleotide in each
21 of the three mutant probes and the wildtype probe;

22 a fourth set of three mutant probes, each identical to
23 the wildtype probe, except in a fourth of the five
24 interrogation positions, which is occupied by a different

25 nucleotide in each of the three mutant probes and the wildtype
26 probe;

27 a fifth set of three mutant probes, each identical to the
28 wildtype probe, except in a fifth of the five interrogation
29 positions, which is occupied by a different nucleotide in each
30 of the three mutant probes and the wildtype probe.

1 94. The array of claim 93 comprising first and second
2 groups of probes, each group as defined by claim 93, the first
3 group comprising a wildtype probe exactly complementary to a
4 first reference sequence, and the second group comprising a
5 wildtype probe exactly complementary to a second reference
6 sequence, wherein the second reference sequence is a mutated
7 form of the first reference sequence.

1 95. The array of claim 94, wherein the first reference
2 sequence is from a CFTR gene and the second reference sequence
3 is a mutated form of the first reference sequence.

1 96. The method of claim 56, wherein the target sequence
2 and the second target sequence are from heterozygous alleles
3 of a CFTR gene.

P53 Chip

1 97. The array of claim 2, wherein the reference sequence
2 is a sequence from a p53 gene.

1 98. The array of claim 2, wherein the reference sequence
2 is from an hMLH1 gene.

1 99. The array of claim 2, wherein the reference sequence
2 is from an MSH2 gene.

1 100. The array of claim 28, wherein the reference
2 sequence is from a human P53 gene and the second reference
3 sequence is from an hMLH1 gene.

1 101. The array of claim 100, further comprising:

2 ninth, tenth, eleventh and twelfth probe sets,

3 (1) the ninth probe set comprising a plurality of
4 probes, each probe comprising a segment of at least three
5 nucleotides exactly complementary to a subsequence of a third
6 reference sequence, the segment including at least one
7 interrogation position complementary to a corresponding
8 nucleotide in the third reference sequence,

9 (2) the tenth, eleventh and twelfth probe sets,
10 each comprising a corresponding probe for each probe in the
11 ninth probe set, the probes in the tenth, eleventh and twelfth
12 probe sets being identical to a sequence comprising the
13 corresponding probe from the ninth probe set or a subsequence
14 of at least three nucleotides thereof that includes the at
15 least one interrogation position, except that the at least one
16 interrogation position is occupied by a different nucleotide
17 in each of the four corresponding probes from the ninth,
18 tenth, eleventh and twelfth probe sets.

1 102. The array of claim 97, wherein the first probe set
2 has at least 60 interrogation positions corresponding to at 60
3 contiguous nucleotides from exon 6.

1 103. The array of claim 98, wherein the reference
2 sequence is exon 5 of a p53 gene, the probes are 17
3 nucleotides long, and the first set of probes is exactly
4 complementary to the reference sequence, and the at least one
5 interrogation position is at position 7, relative to a 3'-end
6 of each probe, which 3'-end is covalently attached to the
7 substrate.

Mitochondrial Chip

1 104. The array of claim 2, wherein the reference
2 sequence is from a mitochondrial genome.

1 105. The array of claim 104, wherein said reference
2 sequence is a sequence of a D-loop region.

1 106. The array of claim 105, wherein D-loop region is
2 full-length.

1 107. The array of claim 104, wherein said reference
2 sequence is at least 90% of a full-length mitochondrial
3 genome.

1 108. The array of claim 104, wherein the reference
2 sequence is bounded by positions 16280 to 356 of the
3 mitochondrial genome.

XX/XX

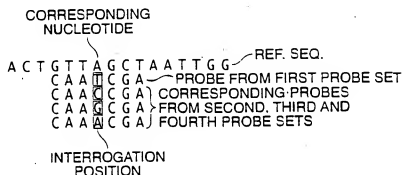


FIG. 1

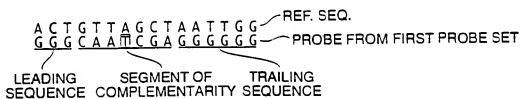


FIG. 2

XX/XX

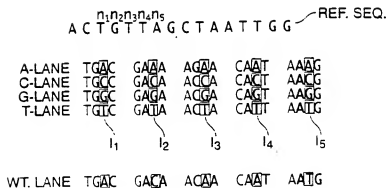


FIG. 4

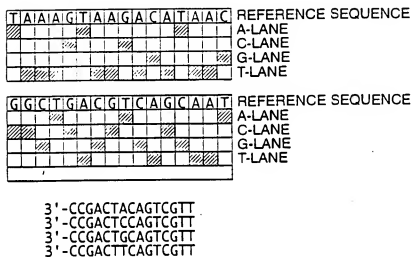


FIG. 5

XX/XX

n CORRESPONDING NUCLEOTIDE
ACTGTTAGCTAATTGG — REF. SEQ.
CAATTCGA — PROBE FROM FIRST SET
CAA—CGATT — DELETION PROBE
CAATTACGA }
CAATTCGA } INSERTION
CAATTCGA } PROBES
CAATTCGA }

FIG. 6

n₁ n₂ n₃ — CORRESPONDING NUCLEOTIDES
A C T G T T A G C T A A T T G G — REF. SEQ.
C A A T T C G A — PROBE FROM FIRST SET
I₁ I₂ I₃ — INTERROGATION POSITIONS

C C A T C G A } CORRESPONDING PROBES
C G A T C G A } FROM SECOND, THIRD AND
C T A T C G A } FOURTH PROBE SETS
I₁

C A A A C G A } CORRESPONDING PROBES
C A A C C G A } FROM FIFTH, SIXTH AND
C A A C C G A } SEVENTH PROBE SETS
I₂

C A A T C A } CORRESPONDING PROBES
C A A T C A } FROM EIGHTH, NINTH AND
C A A T C A } TENTH PROBE SETS
I₃

FIG. 7

XX/XX

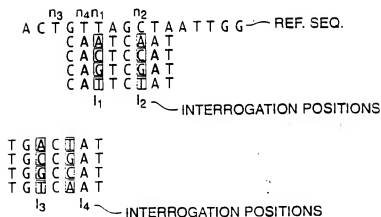


FIG. 8

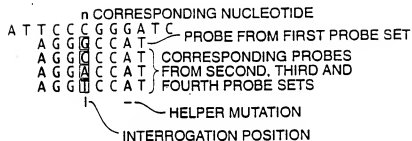


FIG. 9

0.57

HV 407-

30 x 140

19/10/5400

5/7

5/9

7/5

19/10

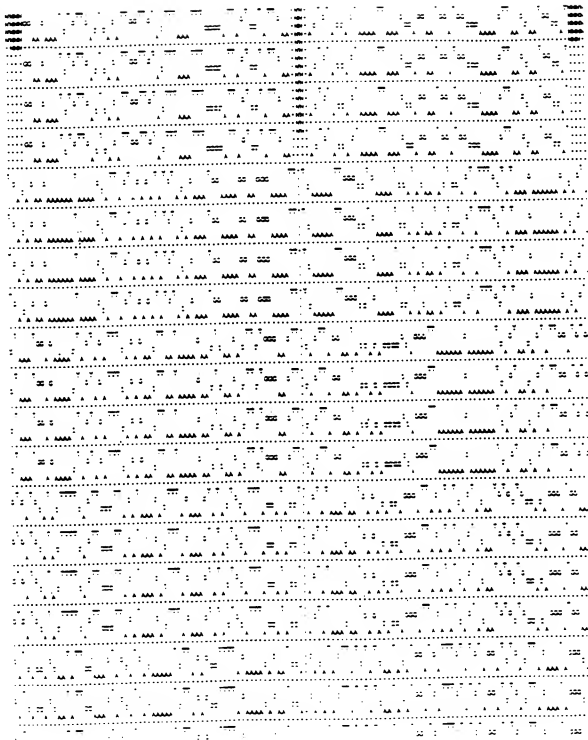


Fig. 10
Page 1 of 2

S. S.

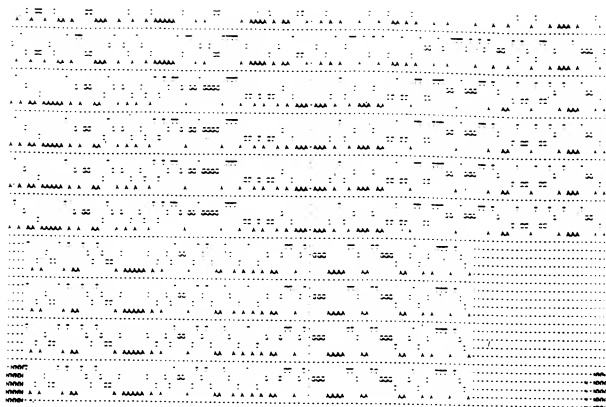
H₂O (2)

Fig. 10
Page 2 of 2



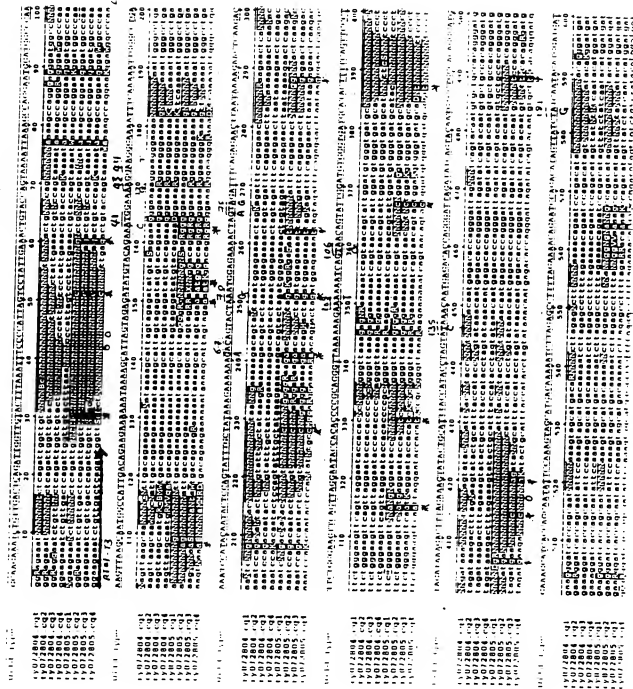
13 probe length
15
17
19

MC07060:

= #07 water chip hybridized with fragmented pFol 19 RNA

Fig. 11

Figure 12
(Page 1 of 2)

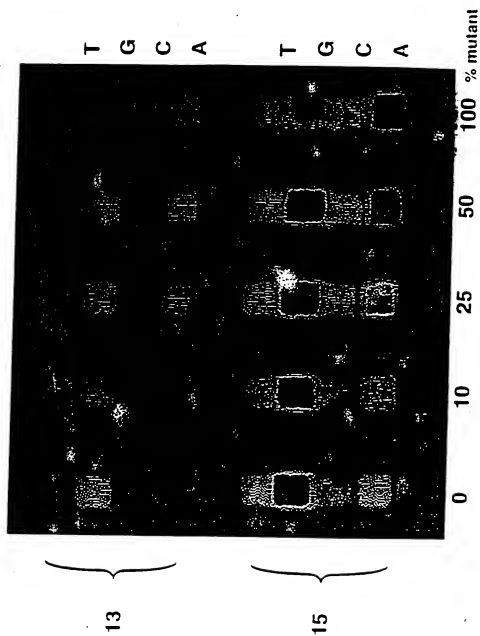


5'Fluorescein-AAAGAAAAAAGACAGTACTAAATGGAGAAAAT wildtype
 PROBE 3' tttttt•tgtcat 13mers
 PROBE 3' cttttttt•tgtcatg 15mers
 PROBE 3' tctttttttt•tgtcatga 17mers
 PROBE 3' ttcctttttt•tgtcatgat 19mers
 5'Fluorescein-AAAGAAAAAAGACAGTACTAAATGGAGAAAAT mutant

Fig. 13

57

Fig. 14



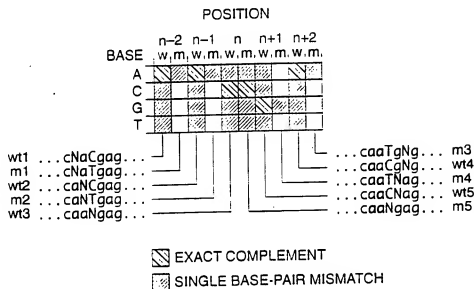
51. 57

Genotyping of HIV-1 Protease Gene
in pre and post-treatment Patients

[illegible][illegible]

↑ MTT ↑
↑ nucleotido
207

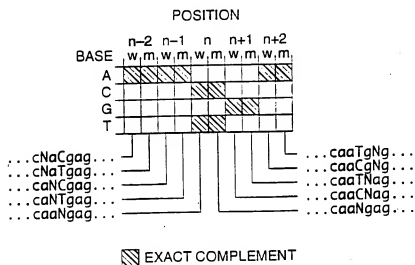
XX/XX



WILD-TYPE SEQUENCE: 5'-AGGTCAA**C**GAGCAA=3'

MUTANT SEQUENCE: 5'-AGGTCAA**T**GAGCAA=3'

FIG. 16



WILD-TYPE SEQUENCE: 5'-AGGTCAA**C**GAGCAA=3'

MUTANT SEQUENCE: 5'-AGGTCAA**T**GAGCAA=3'

FIG. 17

13757

	Probe Sequence	Wild-Type Lane	A-Lane	G-Lane	T-Lane	Target Sequence
A	5'-CATTAAACAAATATCATCTTTCGTTGTTCCCTATG					

B	5'-CATTAAACAAATATCATCTTTCGTTGTTCCCTATG	
---	--	--

C	5'-CATTAAACAAATATCATCTTTCGTTGTTCCCTATG	
---	--	--

Probe set that detects the deletion best

Fig. 18

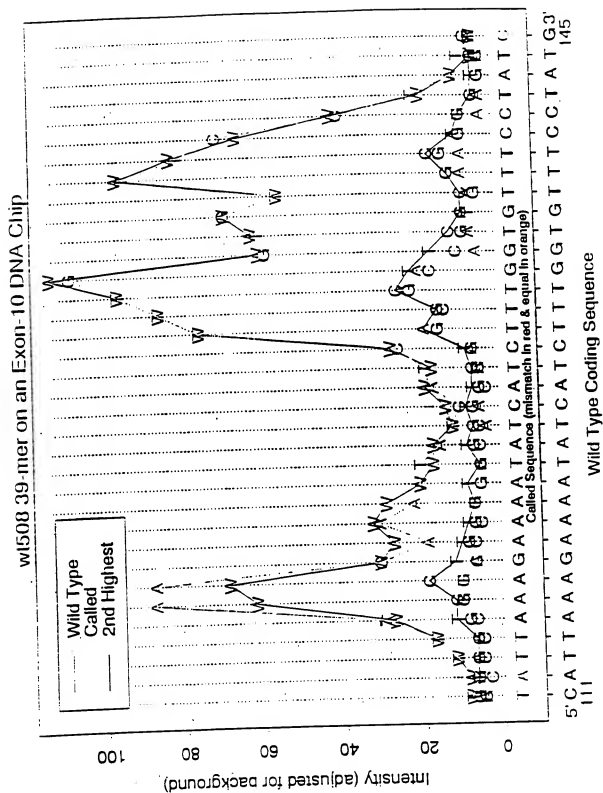


Fig. 19
Page 1 of 3

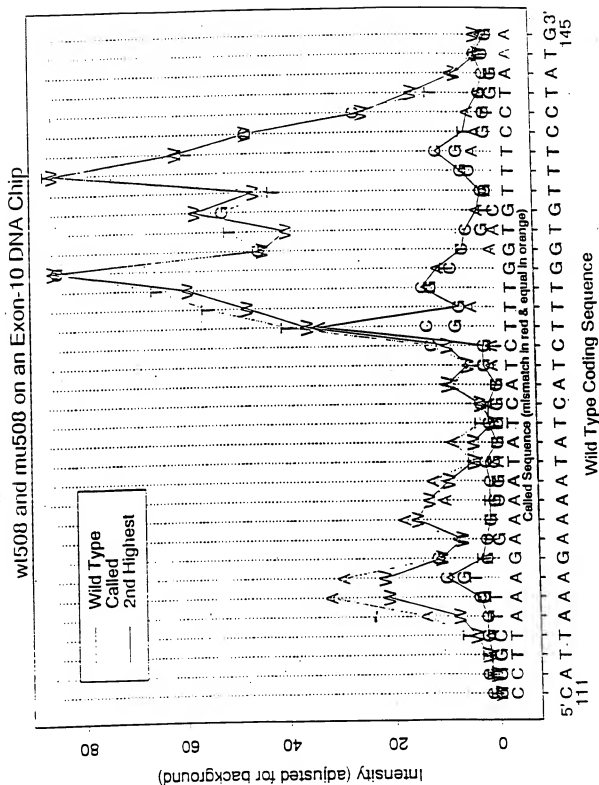


Fig. 19
Page 2 of 3

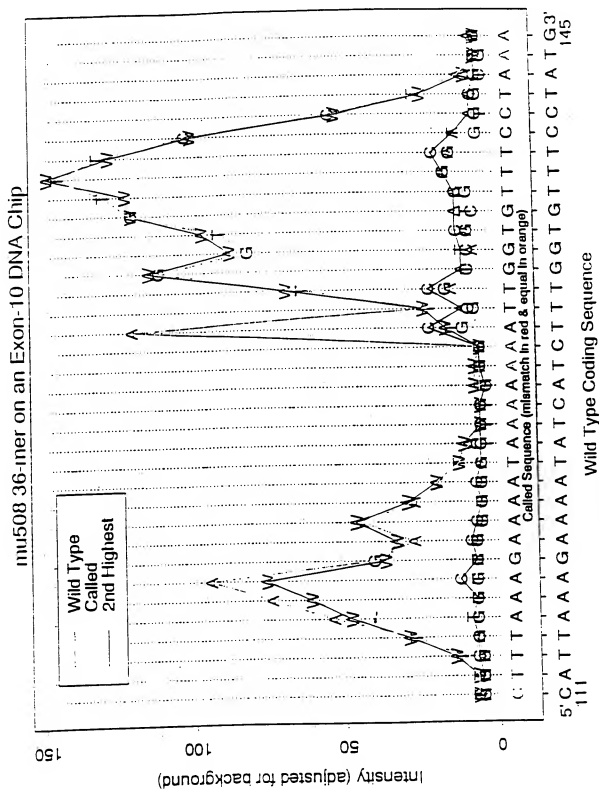


Fig. 19
Page 3 of 3

22/53

Probe Sequence
 Wild-Type Lane
 A-Lane
 C-Lane
 G-Lane
 T-Lane
 Target Sequence

GGAAGTCTCCCATTTTAATT
 5'-CCTTCAGAGGGTAAATTTAA

A

5'-CCTTCAGAGGGTAAATTTAA

B

5'-CCTTCAGAGGGTAAATTTAA

C

Fig. 20

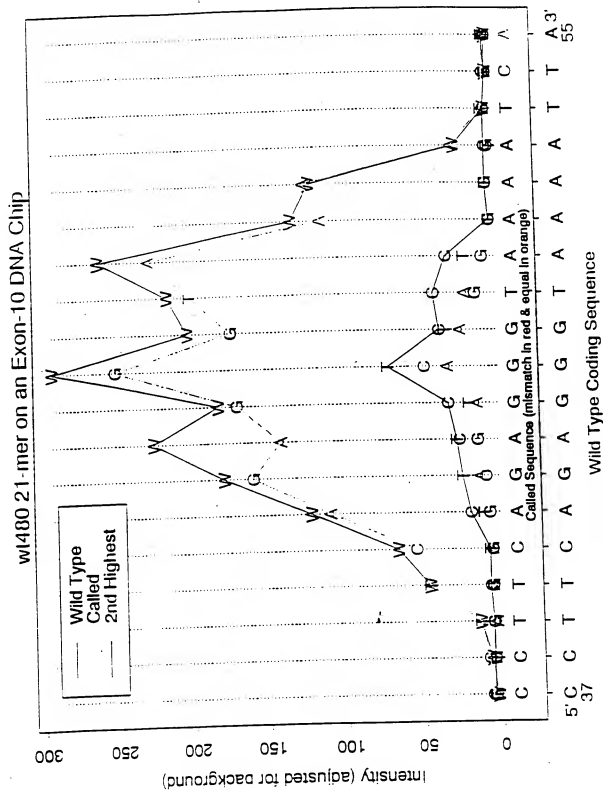


Fig. 21
Page 1 of 3

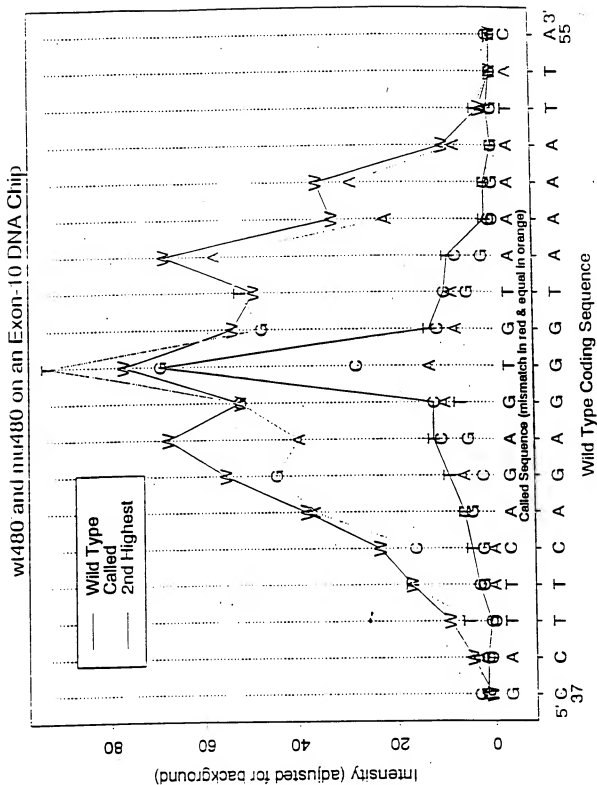


Fig. 21
Page 2 of 3

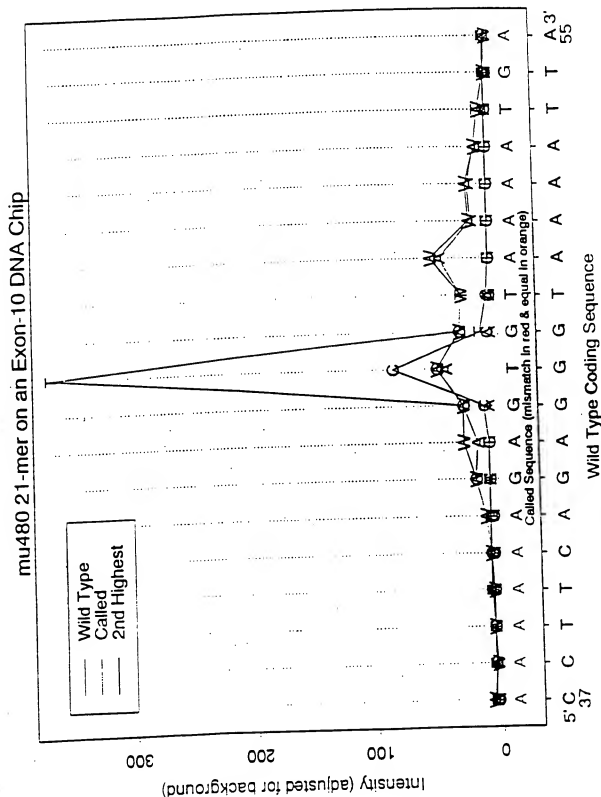


Fig. 21
Page 3 of 3

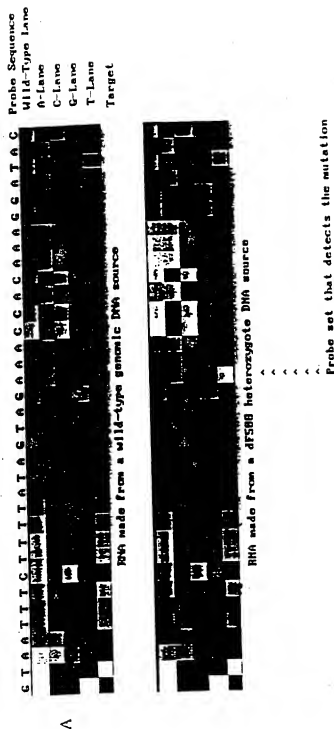


Fig. 22

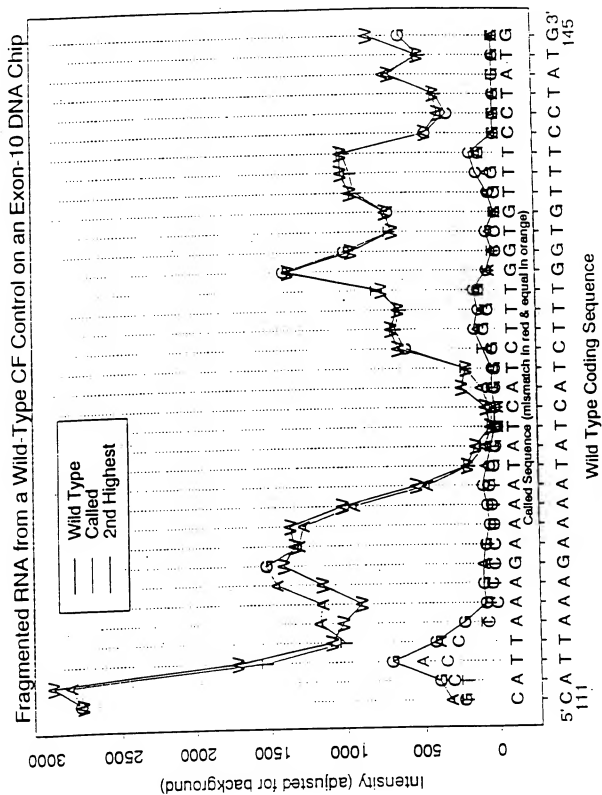


Fig. 23
Page 1 of 2

13:57

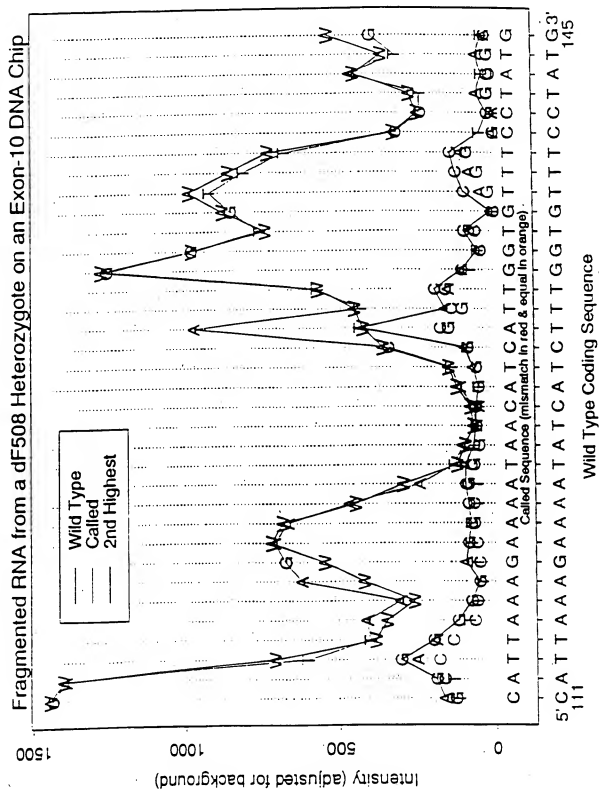


Fig. 23

10.57.

A

B

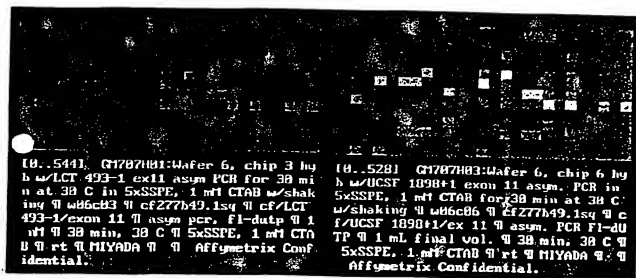


Fig. 24

30 117

A

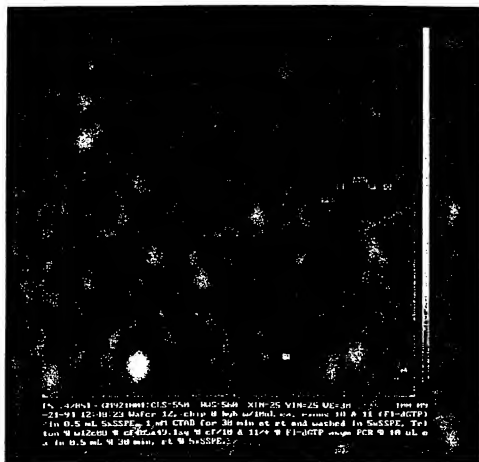


Fig. 25
Page 1 of 2

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B

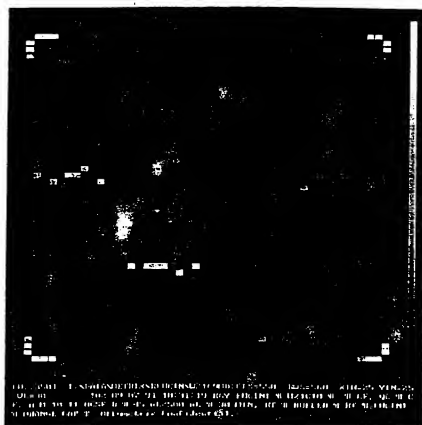


Fig. 25
Page 2 of 2

72 57

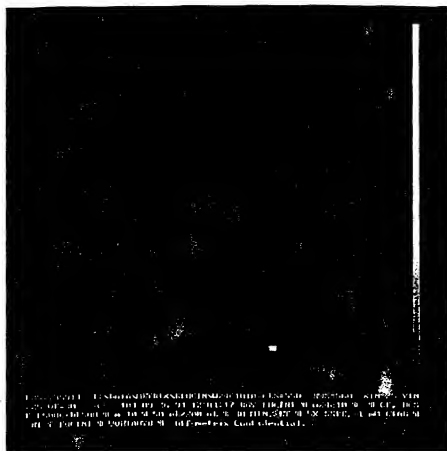


Fig. 26

33/37

P53 EXON 6 CODON 192 REGION: 12MER PROBES

0.	G	A	T	G	C	T	G	A	G	G	A	G
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												

Fig. 27

P53 EXON 6 CODON 192 REGION: 10MER PROBES

0.	G	A	T	G	C	T	G	A	G	G	A	G
1.							C	T	C	C	T	C
2.							A	C	C	T	C	C
3.							G	A	C	T	C	C
4.							C	G	A	C	T	C
5.							A	C	G	A	C	T
6.							T	A	C	G	A	C
7.							C	T	A	C	G	A
8.							T	C	T	A	C	G
9.							T	C	T	A	C	G
10.							A	T	T	C	T	A
11.							T	A	T	C	T	A

Fig. 28

Detection of 12-mer One-Base Substitution P53 Targets

Fig. 29

WT ("G" Substitution)
Target 12-mer



"A" Substitution 12-mer Target



"T" Substitution Target 12-mer



"C" Substitution Target 12-mer



Fig. 31

4:1 Mixture of WT and
"A" Substitution 12-mer
Targets



Figs. 29 and 31

CO, 31

P53 EXON 6 CODON 192 REGION

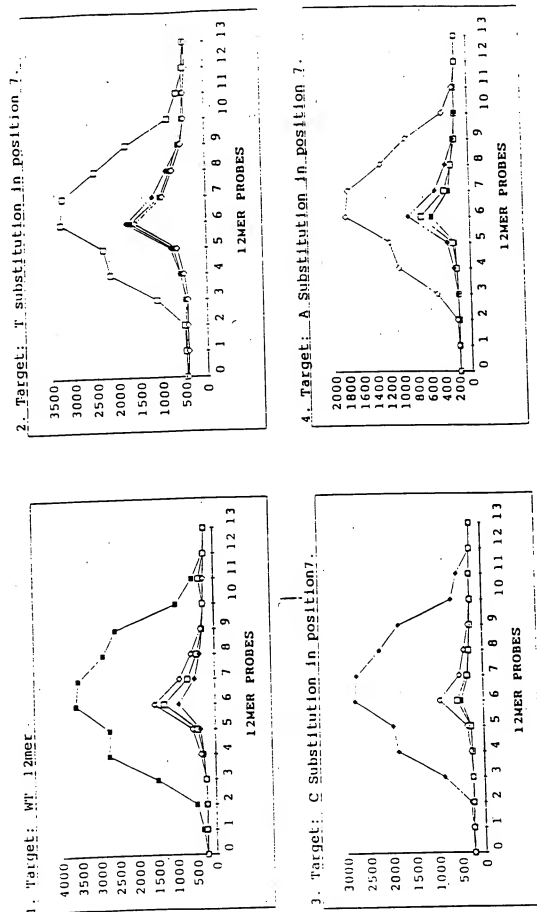


Fig. 30

153 EXON 6 CODON 192 REGION

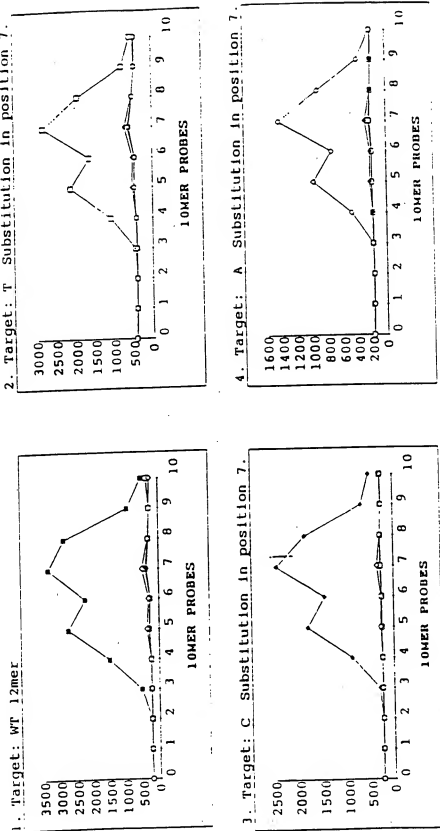


Fig. 32

03, 7

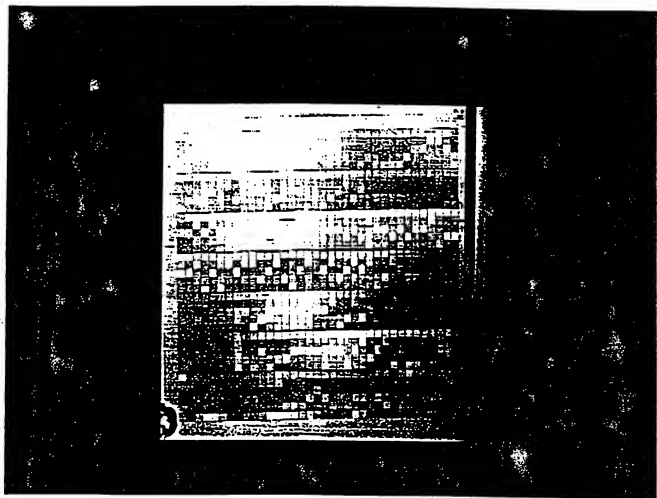


Fig. 33

[illegible]

Fig. 34

- 57 -

THE HUMAN MITOCHONDRIAL GENOME

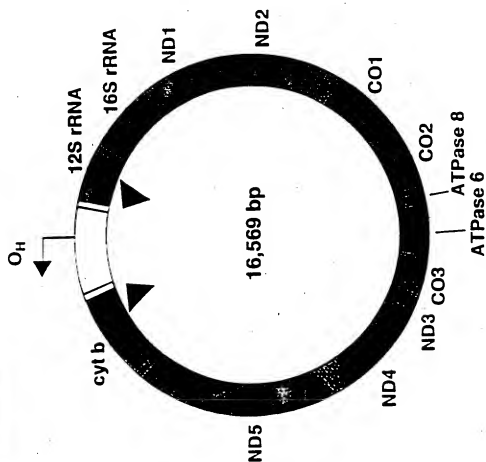
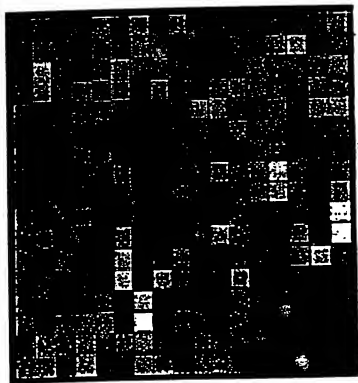


Fig. 35

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mt4

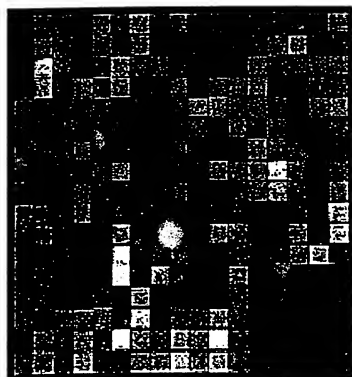


HYBRIDIZATION

Fig. 36

42, 57

mt5



HYBRIDIZATION

Fig. 37

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PREDICTED DIFFERENCE IMAGE

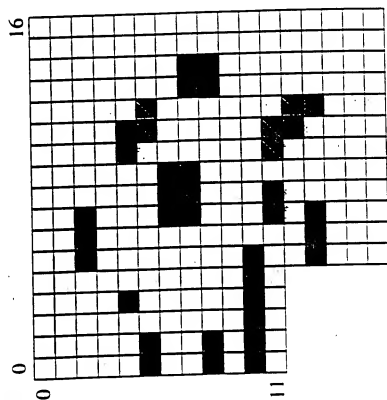


Fig. 38

44, 57

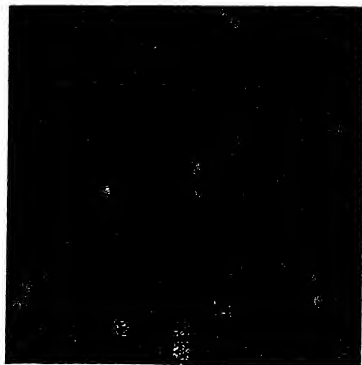
DIFFERENCE IMAGE

Fig. 39

NORMALIZED INTENSITIES

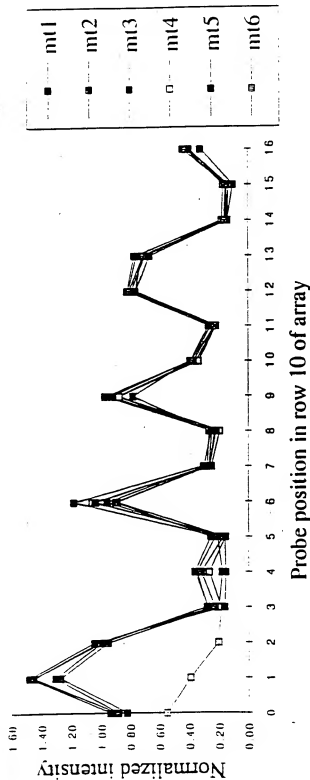


Fig. 40
Sheet 1 of 2

probe position	0	1	2	3	4	5
probe length	13	13	12	12	12	12
sample (mt1 -> 6)	4	4	4	2, 5	2, 5	2, 5
mismatch position from 3' of probe	12	5	3	12	7	2
base change	t->a	t->a	t->a	t->c	t->c	t->c

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NORMALIZED INTENSITIES

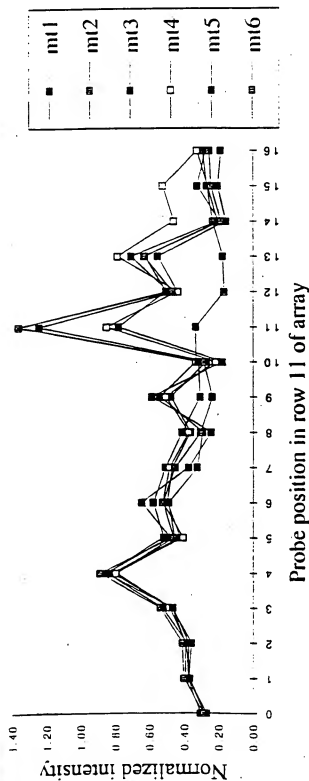


Fig. 40
Sheet 2 of 2

probe position	6	7	8	9	10	11	12	13
probe length	13	12	12	13	14	13	12	12
sample (ml 1 -> 6)	2	2, 5	2, 5, 6	3, 6	3, 4, 5	2, 4, 5	2	2
mismatch position from 3' of probe	13	9, 10	3, 4	11, 5	4, 11, double	11, 3, double	6	3
base change	c -> t	c -> t	c -> t t -> c	t -> c	t -> c double	g -> a t -> c double	g -> a	g -> a

47 57

DISCRIMINATION

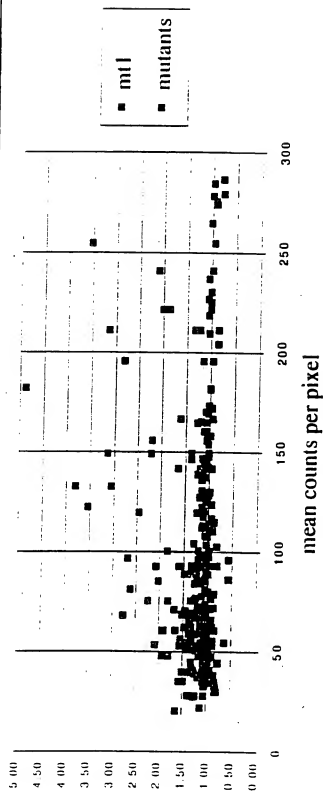


Fig. 41

-3, 57

SEQUENCE & POSITION OF MUTATION

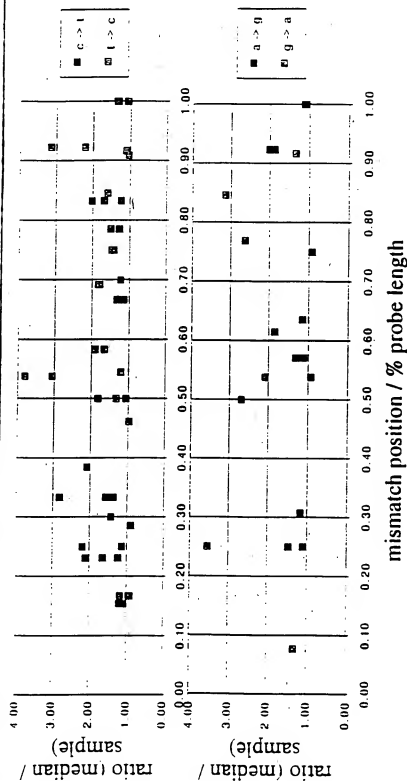


Fig. 42

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SEQUENCE

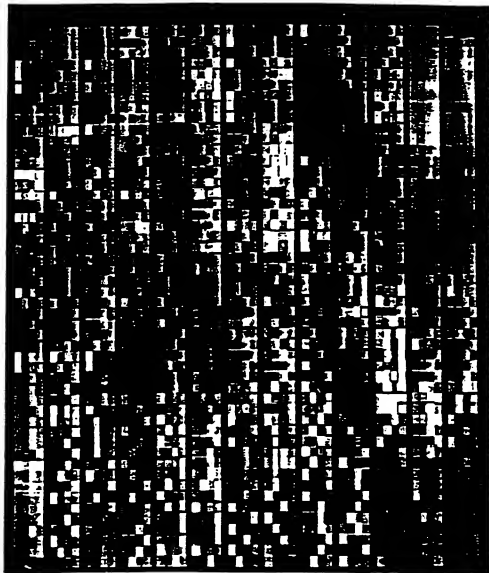
1 XaacaacctaaccaccccttaacagtagtacatagtaacataaagccatttaccX
 50 cgtacatagcacatcacagtcacaatcccttctcgcccccatggatgaccc
 cccctcagatagggtcccttgaccacccatccctccgtgaaatcaataatccc
 gacaaagatgctactctctcgctccgggccccataacacttgggggtag
 claaagtgaactgtatccgacatcttggttccctacttcagggtcataaagc
 claaatagccccacaagttcccccttaataaagacatcacgatggatcacag
 gctatcacccctattaacccactcacgggagctctcccatgcatcttggtatt
 ttcgtctgggggtatgcacgcgatatgcattgcgagacgctggagccgga
 gacccctatgtgcagtatctgtctttgatctctgcctcatctctatttall
 talcgcacctacgttccaatatcacaggcgacaataacttactaaagtgtgt
 taattaatattgcttgtaggacataataataacaatctgaatgtctgcac
 agccActttccacacagacatcataacaaaaatttccaccaaaacccccc
 xctcccccgcttctggccacagcacttaaacacatctctgccaaaccccX

Fig. 43

53 57

Fig. 44

HYBRIDIZATION




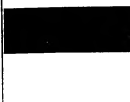


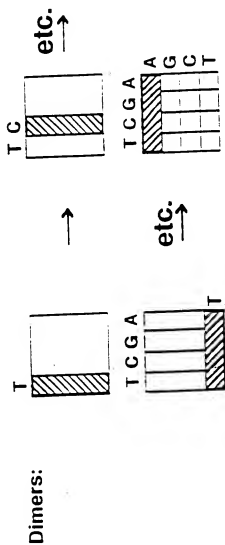
Position:	16519	152	263	344	
Change:	T->C	T->C	A->G	T->C	
Result:					T G C A

Fig. 45

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Nucleoside Combinatorials



in polynomial notation:
 $(T + C + A + G)^2 = \text{All Dimers}$

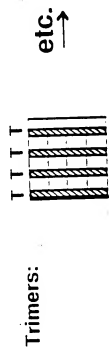
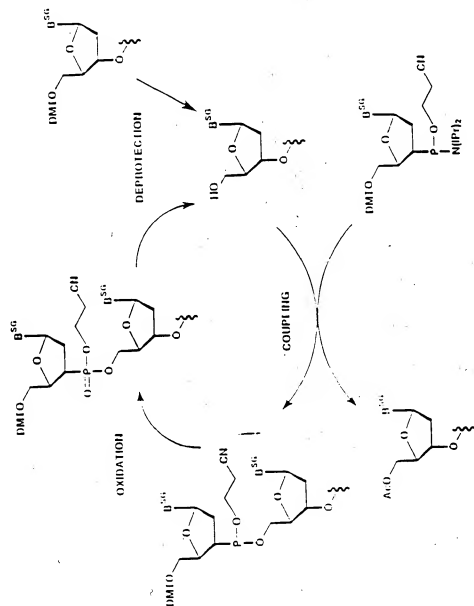


Fig. 47

4-57

Fig. 48

Solid Phase DNA Synthesis

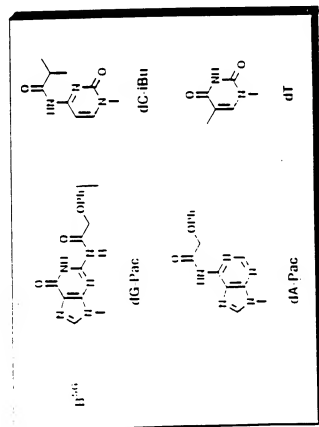
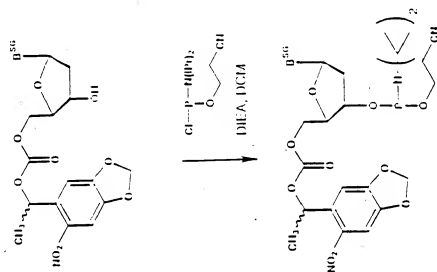


Nucleoside Buildingblocks



(13)
(14)
(15)

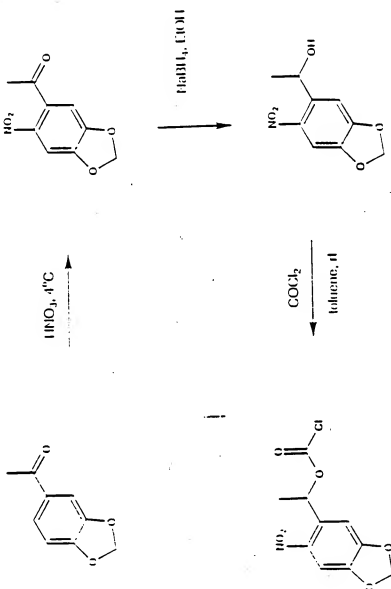
Fig. 49



58

Fig. 50

MeNPOC-Cl



Detection

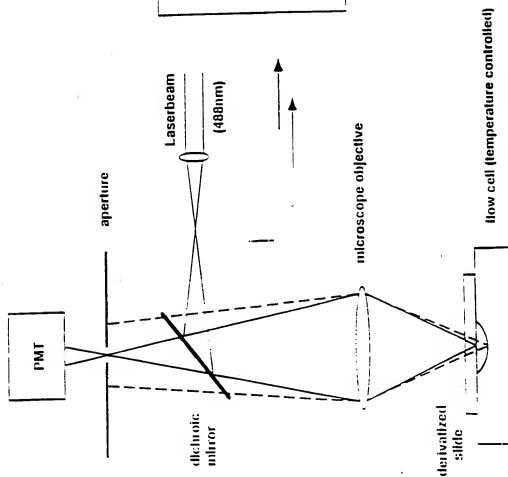


Fig. 51